Excerpt From:
Diversity of Marine and Freshwater Algal Toxins

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Diarrhetic ShellfishToxins

The diarrhetic shellfish toxins (DTX) are a class of acidic polyether toxins produced by dinoflagellates and responsible for human illness, diarrhetic shellfish poisoning (DSP), associated with seafood consumption. This toxin class consists of at least eight congeners including the parent compound, okadaic acid, which was first isolated from the black sponge, Halichondria fortii (70) (Figure 9). Okadaic acid, DTX-1 and DTX-2 are the primary congeners involved in shellfish poisoning, with the other congeners believed to be either precursors or shellfish metabolites of the active toxins. DSP is widespread in its distribution (Figure 10), with essentially seasonal occurrences in Europe and Japan. The first incidence of human shellfish-related illness identified as DSP occurred in Japan in the late 1970's, where the dinoflagellate Dinophysis fortii was identified as the causative organism, and the toxin responsible was termed dinophysistoxin (DTX-1) (71,72). Retrospective analysis of similar disease outbreaks in the Netherlands (73,74) and Scandanavia (75) confirmed that these were also associated with Dinophysis. The major toxins involved in European outbreaks are okadaic acid and DTX-1. However, incidents in Ireland and Portugal were found to include an additonal toxin, DTX-2 (76). The first confirmed incident of DSP in North America occurrend in 1990 in the maritime provinces of Canada (77), but was associated with the benthic dinoflagellate Prorocentrum lima, and two toxins, DTX-1 and okadaic acid. Okadaic acid and related DTX toxins are also produced by a number of other Prorocentrum species, including P. maculosum, P. concavum, P. hoffmanianum, but do not appear to be elaborated by P. micans, P. minimum, or P. mexicanum (78).

The DTXs are inhibitors of ser/thr protein phosphatases. Inhibitory activity against protein phosphatases is specific for classes PP2A (okadaic acid IC50 ~ 0.5 nM) and PP1 (okadiac acid IC50 ~ 50 nM), with PP2B being inhibited only at high concentrations (okadiac acid IC50 > 10mM) and PP2C being insensitive. The binding site for okadaic acid resides on the catalytic subunit of the protein phosphatase, at the active site of the ezyme, as determined by x-ray crystal structures (79), molecular modeling (80) and mutational analyses (81). Binding to this site requires the carboxylic acid moiety, which accounts for the inactive state of the diol esters and DTX-4.

Ser/thr protein phosphatases are critical components of signaling cascades in eukaryotic cells which regulate a diverse array of cellular processes involved in metabolism, ion balance, neurotransmission, and cell cycle regulation (82). Diarrhea associated with DSP is most likely due to the hyperphosphorylation of proteins, including ion channels, in the intestinal epithelia (83), resulting in impaired water balance and loss of fluids. In addition, okadaic acid-like polyether toxins have been identified as tumor promotors (84,85). The toxic potency of okadaic aicid is much lower than that of the neurotoxin polyethers, with an LD50 of 192 mg/kg (i.p.) in mice (86).

The biosynthesis of the DTX toxins and the mechanisms by which the dinoflagellate protects itself from its toxins have received much attention. Okadaic acid is localized to the chloroplast (87). Most of the intracellular toxin in P. lima is present in the form of DTX-4 (88), which has been shown in metabolic labeling studies to be a biosynthetic precursor to okadaic acid (89). Thus, it is proposed that okadaic acid is released by P. lima as the inactive pro-toxin, DTX-4, which is reduced extracellularly to the diol ester in the medium of P. lima. The diol ester may then be cleaved at the ester linkage to yield the active toxin, okadaic acid. Windust et al (90,91) investigated the allelopathic activity of okadaic acid against other species of microalgae and found that it could inhibit growth of diatoms at mM concentrations. However, Sugg and Van Dolah (92) tested the hypothesis that okadaic acid produced by P. lima confers competitive advantage over co-occurring benthic dinoflagellates of the ciguatera assemblage, and found that the observed growth inhibition of other dinoflagellates by P. lima was not attributable to okadaic acid. Dinoflagellates do possess okadiac acid-sensitive protein phosphatases PP1 and PP2A (92,93,94), whereas the protein phosphatases in P. lima, appear to be insensitive.

References:

70. Tachibana, K, PJ Scheuer, Y Tsukitani, H Kikuchi, D Van Engen, J Clardy,Y Gopichand, FJ Schmitz. Okadaic acid, a cytotoxic polyether from two marine sponges of the genus Halichondria. J Am Chem Soc 103: 2469-2471, 1981.

71. Yasumoto, T, Y Oshima, M Yamaguchi. Occurrence of a new type of shellfish poisoning in Japan and chemical properties of the toxin. In: D Taylor, HH Seliger, eds. Toxic Dinoflagellate Blooms. Amsterdam: Elsevier, 1979, pp. 495-502.

72. Yasumoto, T, Y Oshima,W Sugawara, Y Fukuyo, H Oguri, T Igarishi, N Fujita. Identification of Dinophysis fortii as the causative organism of diarrhetic shellfish posioning. Bull Jpn Soc Sci Fish 46: 1405-1411, 1980.

73. Kat, M. Occurrence of Prorocentrum species and coincidental gastrointestinal illness of mussel consumers. In: D Taylor, HH Seliger, eds. Toxic Dinoflagellate Blooms. Amsterdam: Elsevier, 1979, pp.215-220, 1979.

74. Kat, M. Dinophysis acuminata blooms, the distinct cause of Dutch mussel poisoning. In: DM Anderson, AW White, DG Baden, eds. Toxic Dinoflagellates. Amsterdam: Elsevier, 1985, pp.73-77.

75. Kumagai, M, T Yanagi, M Murata,T Yasumoto, M Kat, P Lassus, JA Rodriguez-Vazquez. Okadaic acid as the causative toxin of diarrhetic shellfish poisoning in Europe. Agric Biol Chem 50: 2853-5857, 1986.

76. Hu, T, J Doyle, D Jackson, J Marr, E Nixon, S Pleasance, MA Quilliam, JA Walter JLC Wright. Isolation of a new diarrhetic shellfish toxin from Irish mussels. Chem Commun 39-41, 1992.

77. Quilliam, MA, M Gilgan, S Pleasance, ASW Defrietas, D Douglas, L Fritz, T Hu, JC Marr, C Smyth, JLC Wright. Confirmation of an incident of diarrhetic shellfish poisoning in eastern Canada. In: TJ Smayda, Y Shimizu, eds. Toxic Phytoplankton Blooms in the Sea. Amsterdam: Elsevier, 1993, pp. 547-552.

78. Morton, SL, DR Tindall, D.R. Determination of okadaic acid content of dinoflagllate cells: a comparison of the HPLC-fluorescent method and two monoclonal antibody ELISA test kits. Toxicon 34: 947-954, 1996.

79. Gauss, C-M, JE Sheppock, AC Nairn, RA Chamberlin. A molecular modeling analysis of the binding interactions between okadaic acid class of natural product inhibitors and the ser-thr phospharases, PP1 and PP2A. Bioorganic and Medicinal Chem 5: 1751-1773, 1997.

80. Bagu, JR, BD Sykes, MM Craig, CFB Holmes. A molecular basis for different interactions of marine toxins with protein phosphatase –1. Molecular models for bound moruporin, microcystins, okadaic acid and calyculin A. J Biol Chem 272: 5078-5097, 1997.

81. Zhang, Z, S Zhao, F Long, L Zhang, G Bai, H Shima, M Nagao, EYC Lee. A mutant of protein phosphatase-1 that exhibits altered toxin sensitivity. J Biol Chem 269: 16997-17000, 1994.

82. Wera , S, B Hemmings. Ser/thr protein phosphatases. Biochem J 311: 17-29, 1995.

83. Cohen, P, CFB Holmes, Y Tsukitani. Okadaic acid: a new probe for the study of cellular regulation. TIBS 15: 98-102, 1990.

84. Sugunama, K, H Fujiki, H Sugur, S Yoshizawa, M Hirota, M Nakayasu, M Ojika, K Wakamatsu, K Yamada, T Sigimura. Proc. Nat. Acad. Sci. 85: 1768-1771, 1988.

85. Fujiki, H, K Sugunama, H Suguri, S Yoshizawa, K Tagai, N Uda, K Wakamatsu, K Yamada, M Murata, T Yasumoto, T Sugimura. Diarrhetic shellfish toxin, dinophysistoxin-1, is a potent tumor promotor on mouse skin. Jpn J Cancer Res 79:1089-1093, 1988.

86. Yasumoto, T, M Murata, Y Oshima, G Matsumoto, J Clardy. Diarrhetic shellfish poisoning. In: EP Regalis, ed. Seafood Toxins. Washington, D.C.: American Chemical Society, 1984, pp. 207-214.

87. Zhou, J, L Fritz. Okadaic acid localizes to the chloroplasts in DSP-toxin-producing dinoflagellates, Prorocentrum lima and Prorocentrum maculosum. Phycologia 33: 455-461, 1994.

88. Quilliam, MA, NW Ross. Analysis of disrrhetic shellfish poisoning toxin and metabolites in plankton and shellfish by ion-spray liquid chromatography-mass spectrometry. In, Biochem. and Biotech. of Electrospray Ionization Mass Spectrometry, 1996, pp. 351-364.

89. Needham, J, T Hu, JL MacLachlan, JA Walter, JLC Wright. Biosynthetic studies of the DSP toxin DTX-4 and an okadaic acid diol ester. J. Chem Soc Chem Comm, 1623-1624, 1995.

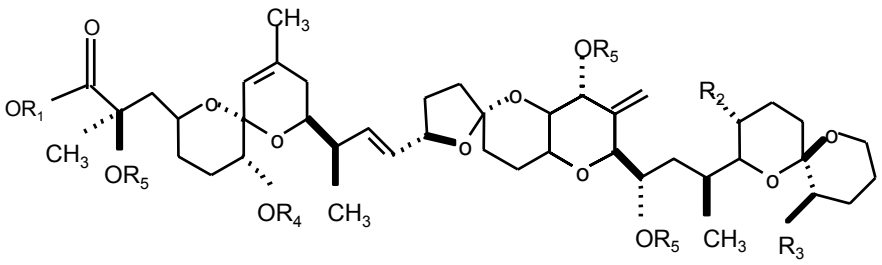
90. Windust, AJ, JLC Wright, JL MacLachlin. The effects of the diarrhetic shellfishpoisoning toxins, okadaic acid and dinophysistoxin-1, on the growth of microalgae. Mar. Biol. 126: 19-25, 1996.

91. Windust, AJ, MA Quilliam, JLC Wright, JL MacLachlin Comparative toxicity of the diarrhetic shellfish poisons, okadaic acid, okadaic acid diol-ester and dinophysistoxin-1, to the diatom Dinophysis weissflogii. Toxicon 35: 1591-1603, 1997.

92. Sugg, LM, FM Van Dolah. 1999. No evidence for an allelopathic role of okadaic among ciguatera associated dinoflagellated. J Phycol 35:93-103.

93. Boland, MP, MFJR Taylor, CFB Holmes. Identification and characterization of type-1 protein phosphatases from the okadaic acid producingmarine dinoflagellate, Prorocentrum lima. FEBS 334: 13-17, 1993.

94. Boland MP, CFB Holmes.Identification and characterization of protein phosphatase-1 and –2A in marine dinoflagellates. Adv Prot Phosphatases 8: 325-342, 1994



	R1	R2	R3	R4	R5
OA	H	CH3	H	H	H
DTX-1	H	CH3	CH3	H	H
DTX-2	H	H	CH3	H	H
DTX-3	H	CH3	CH3	acyl	H
DTX-4	1	H	H	H	H
DTX-5a	2	H	H	H	H
DTX-5b	3	H	H	H	H
Diol ester	4	H	H	H	H
OA methyl ester	CH3	CH3	H	H	H

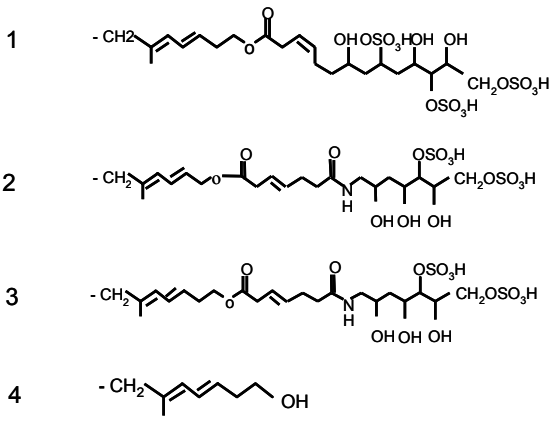


Figure 9. Structures of naturally occurring DSP toxins.

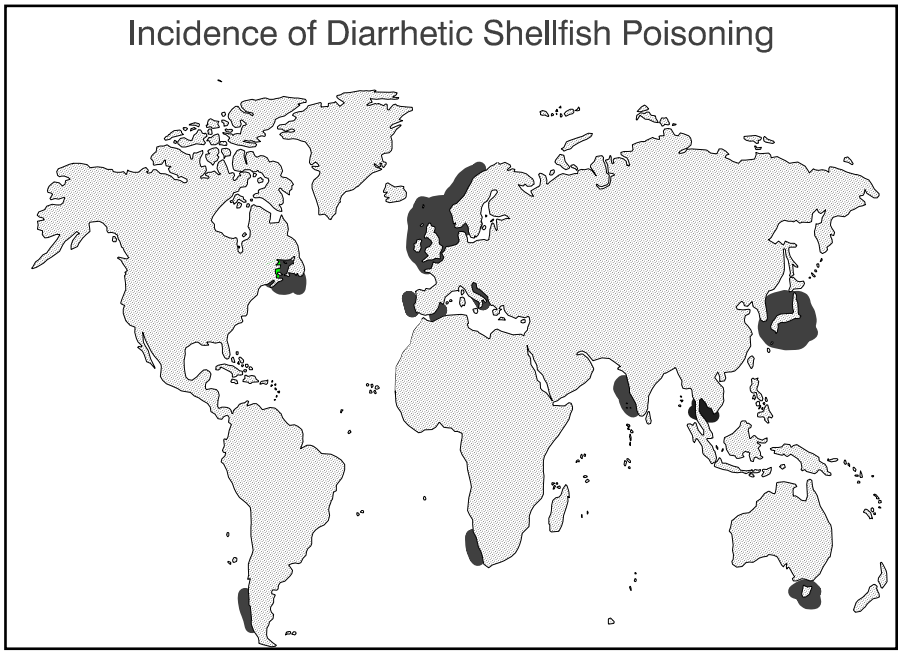


Figure 10. Distribution of diarrhetic shellfish poisoning.